OCCURRENCE OF α-HYDROXY FATTY ACIDS IN *ACTINOMYCETALES*

Ikuya YANO, Yoshiya FURUKAWA and Masamichi KUSUNOSE

Research Laboratory of Biochemistry, Toneyama Hospital, National Sanatorium, and Toneyama Institute for Tuberculosis Research, Osaka City University, Toyonaka, Osaka, Japan

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1. Introduction

Long-chain α-hydroxy fatty acids are well known to predominate in cerebroside and its related lipids in animals and yeasts. In contrast, little attention had been paid to the occurrence and metabolism of α-hydroxy fatty acids in bacteria. Recently, Asselineau and co-workers [1,2] found that α-hydroxy anteiso-pentadecanoic acid is present in the state of free fatty acid in *Streptomyces*. Subsequently, Kawanami and co-workers [3,4] reported that phosphatidylethanol-amine from *Streptomyces sioyaensis* contained α-hydroxy iso-pentadecanoic acid at the β-position of the glycerol moiety. During the course of our study on the fatty acids of *Nocardia*, we have also found α-hydroxy anteiso-pentadecanoic acid to be present as the component of the phosphatidylethanolamine from *Nocardia leishmanii* [5]. This finding led us to survey the distribution of α-hydroxy fatty acids in bacteria. On the basis of thin-layer and gas-liquid chromato-graphic, and mass spectrometric analysis, the present paper describes that various α-hydroxy fatty acids occur abundantly in four different species belonging or closely related to *Actinomycetales*.

2. Materials and methods

Nocardia leishmanii, Waksmania rosea and *Streptosporangium roseum*, and *Arthrobacter simplex* were kindly supplied by Dr. M.Mayama, Shionogi Research Laboratories, Osaka and Prof. S.Fukui, Department of Industrial Chemistry, Kyoto University, Kyoto, respectively. The medium contained 1% glucose, 1% peptone and 0.5% yeast extract (Difco), with pH adjusted to 7.0. The cells were incubated at 30° for 4 days on a rotary shaker. After the cells were harvested by centrifugation, lipids were extracted with chloroform-methanol (2:1, v/v) and washed by the method of Folch et al. [6], then hydrolysed with 10% methanolic KOH for 1 h. Unsaponifiable materials were removed with diethyl ether, and after acidification with 1 N HCl, total fatty acids were extracted and transmethylated with 5% HCl-methanol for 2 h under reflux. The mixture of fatty acid esters thus obtained was separated into polar and non-polar fatty acid esters on a thin-layer plate of silica gel H (Merck) with a solvent of light petroleum (b.p. 35–60°)-diethyl ether (85:15, v/v). Gas-liquid chromatographic analysis was carried out using a Packard Model 800 instrument equipped with argon ionization detector. The glass-coiled column packed with 15% ethylene glycol adipate polyester on Chromosorb W was operated at 185°. Mass spectra were taken with a Hitachi RMU mass spectrometer. The energy of the bombarding electrons was 80 eV and the ionization current 80 μamp. The temperature of the ion source was 250°. α-Hydroxy fatty acids for standard were purchased from Applied Science Lab., Pa., USA, or synthesized from non-substituted fatty acids [7].

3. Results and discussion

Each thin-layer chromatography of the mixed fatty acid methyl esters obtained from four species described above gave two principal spots, one corresponding to non-polar fatty acid esters, and the other

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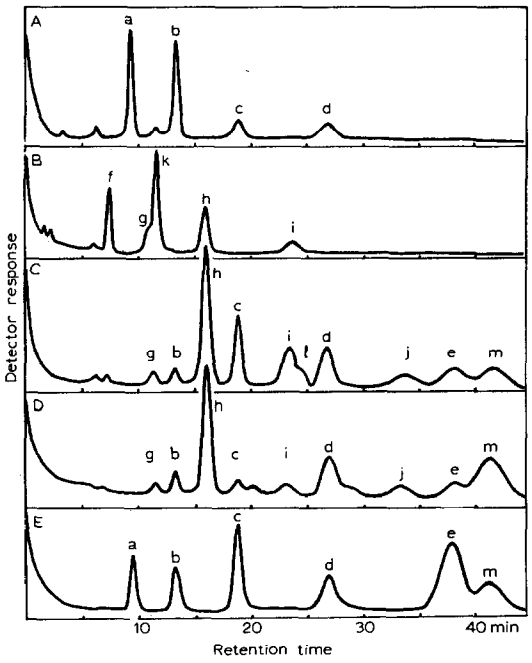

Fig. 1. Gas-liquid chromatograms of methyl esters of α-hydroxy fatty acids from *Waksmania rosea* (A), *Nocardia leishmanii* (B), *Arthrobacter simplex* (C) and *Streptosporangium roseum* (D). Each sample was recovered from the spot of the polar fatty acid esters on a thin-layer plate, and submitted to gas-liquid chromatography, then compared with authentic α-hydroxy fatty acid esters (E) in retention time.

being similar in migration to α-hydroxy fatty acid ester standard. Under the condition employed here, α-hydroxy fatty acid esters can be apparently differentiated from other positionally isomeric hydroxy fatty acid esters [8]. Such polar fatty acid esters comprised approx. 3, 10, 15 and 45% of the total fatty acid esters of *Waksmania rosea, Streptosporangium roseum, Nocardia leishmanii* and *Arthrobacter simplex*, respectively.

For further identification, the polar fatty acid esters were recovered from each plate and submitted to gas-liquid chromatography. Fig. 1 illustrates that the polar fatty acid esters from each species consist of five or more components. From comparison of log retention time with authentic samples, homologous series of straight chain α-hydroxy fatty acids were identified as follows: α-OH *n*-C_{14} for (a), α-OH *n*-CH_{15} for (b), α-OH *n*-C_{16} for (c), α-OH *n*-C_{17} for (d), α-OH *n*-C_{18} for (e). On the other hand, based on the parameters of Miwa et al. [9], homologous series of branched-chain α-hydroxy fatty acids were tentatively identified as follows: α-OH iso-C_{14} for (f), α-OH iso-C_{15} for (g), α-OH iso-C_{16} for (h), α-OH iso-C_{17} for (i), α-OH iso-C_{18} for (j), α-OH anteiso-C_{15} for (k), α-OH anteiso-C_{17} for (l). Peak (m) was tentatively identified as α-hydroxytuberculostearic acid, because its retention time was identical with that of α-hydroxy-10(or 9)-methylstearic acid ester synthesized by catalytic hydrogenation of α-hydroxysterculic acid obtained from *Pachira insignis* seed oil (Tropical Institute, London, U.K.) [10].

These fatty acid esters were further characterized by mass spectrometry after trapping of the individual effluents. Each of the major fatty acid esters gave a peak at m/e 90 and 103, which corresponds to the fragment obtained from 2-hydroxy methyl ester, in addition to each parent peak indicating a molecular weight. Acetylated fatty acid esters also gave a peak at m/e 132 due to 2-acetoxy methyl ester. A typical pattern of mass spectra of acetylated component (h) from *A. simplex* (fig. 1) is shown in fig. 2. Peaks at M = 328, M = 15 (due to loss of methyl branch), M − 42 (due to loss of ketene), M − 59 (a), m/e 132 and m/e 90 are prominent, thus indicating that this compound is methyl 2-acetoxy-14-methylpentadecanoate. A peak at m/e 43 (isopropyl radical) is much higher than a peak at m/e 57 (isobutyl radical), supporting the structure of iso-methyl branching rather than that of anteiso-methyl branching. In the infrared spectrum of the major components, a hydroxy group in the α-position was again indicated by a wide-strong band at 2.8–2.9 μ. Furthermore, gas-liquid chromatography of non-polar fatty acid esters obtained after reduction of polar fatty acid esters with HI, red phosphorus and zinc dust under reflux overnight gave non-substituted fatty acid esters with carbon skeleton corresponding to the original hydroxy fatty acids.

These results indicate that various types of long-chain α-hydroxy fatty acids are distributed in several species of *Actinomycetales* and related groups.

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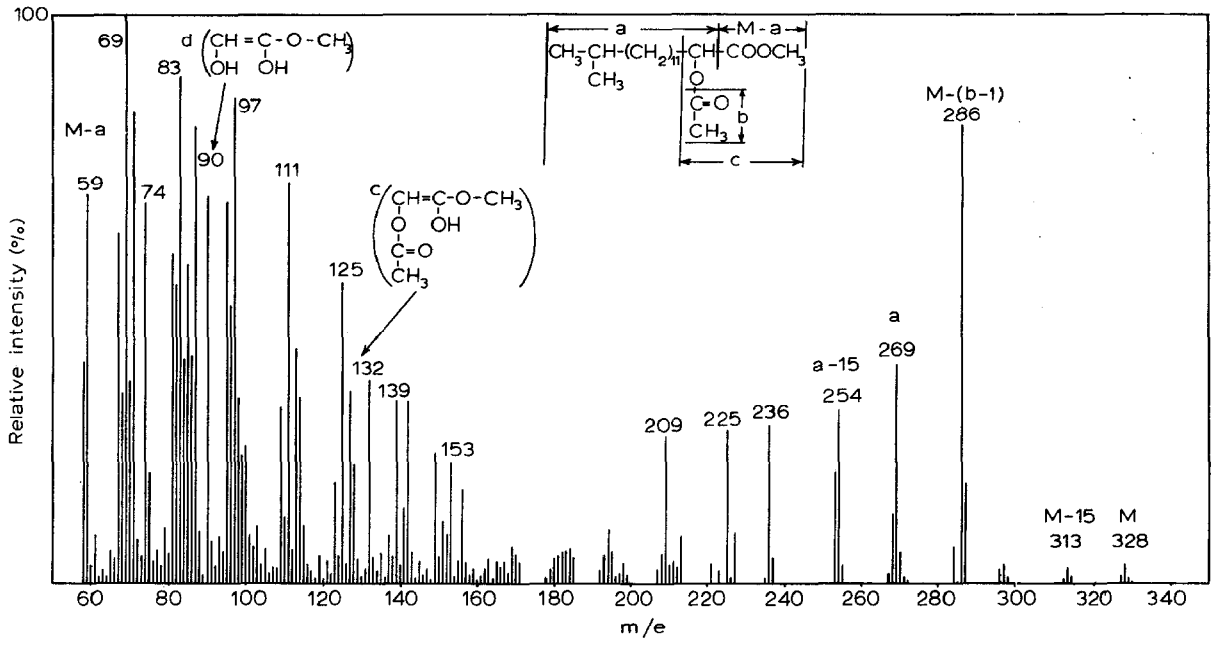

Fig. 2. Mass spectra of methyl ester of the 2-acetoxy-14-methyl-pentadecanoic acid (component (h) in fig. 1) from *Arthrobacter simplex*. Upon gas-liquid chromatography, the methyl ester of α-hydroxy iso-C_{16} acid was collected, and then acetylated with acetic anhydride in pyridine.

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